CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Artificial Sweeteners by Pulsed Amperometric Detection

Purpose
Determination of artificial sweeteners and other polyalcohols by pulsed amperometric detection (PAD) at a gold electrode.

LC determination of polyalcohols and carbohydrates is of interest to the food industry. These compounds are easily separated by either reverse-phase or ion-chromatography techniques, but lack a chromophore that would allow the use of a UV-Visible detector [1]. They are electroactive, but in most cases the electrode becomes coated with oxidation products that impede detection (but see reference [2]). Pulsed amperometric detectors apply a waveform that alternates cleaning and regenerating potentials with sampling potentials, to remove any reaction products which may foul the electrodes [3, 4].

Existing Methods
Liquid chromatography with refractive index detection, which suffers from lack of sensitivity and selectivity.

Conditions
System: BAS 480 Liquid Chromatograph with the DA-5 Data Analysis and Control system
Detector: BAS LC-4C Amperometric Detector in the PAD mode
Electrode: 3 mm gold (MF-1002)
Reference Electrode: Ag/AgCl (MF-2021)
Waveform: See T1
Column: Hamilton RCX-30 anion exchange, 150 x 4.6 mm, 7 µm particle size
Mobile Phase: 100 mM NaOH
Flow Rate: 1.5 mL/min
Injection Volume: 5 µL
Linear Range: 200 pg to at least 100 ng

Sample Preparation
About 1 g of toothpaste or coarsely shredded chewing gum was stirred in water for 30 min, diluted to 100 mL with water, filtered through a 0.45 µm filter, then diluted another 100 times with water. Total dilution = 10,000 times. It is likely that only the surface polyalcohols were sampled from the gum samples.

Table 1. Waveform for detection of carbohydrates and polyalcohols. * indicates the sampling step.

Interval (msec)	Potential (mV *vs.* Ag/AgCl)
400	50
200	50 *
200	800
200	-600

Notes
A chromatogram of standard glycerol, xylitol and sorbitol is shown in F1. The toothpaste and gum samples are shown in F2-F4.

Oxidation of carbohydrates and polyalcohols at a gold electrode occurs only at high pH [3, 4]. This allows the use of ion-exchange chromatography, as carbohydrates become anionic at high pH. The mobile phase must be continuously sparged with helium, and all plumbing must be stainless steel, because CO_2 from the air will form carbonates that affect column performance. Due to this fact, the column manufacturer recommends the use of carbonate-free NaOH.

Since alkaline mobile phases will attack glass, the solvent reservoir must be an inert plastic, such as high-density polyethylene (HDPE) or polyester (PETE).

The high-pH mobile phase will affect the appearance of the reference electrode, turning its clear gel filling to an opaque green. This did not affect the performance of the reference electrode over several weeks of testing.

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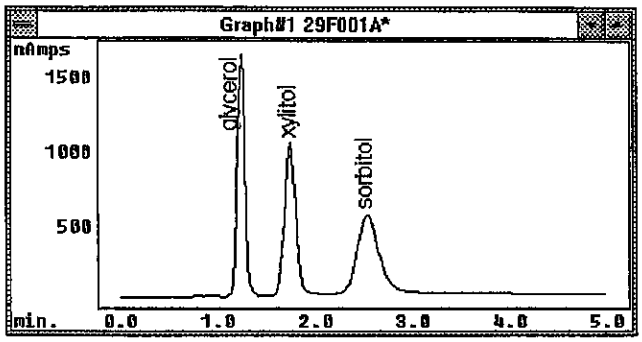

Figure 1. Separation of 100 ng each glycerol, xylitol and sorbitol.

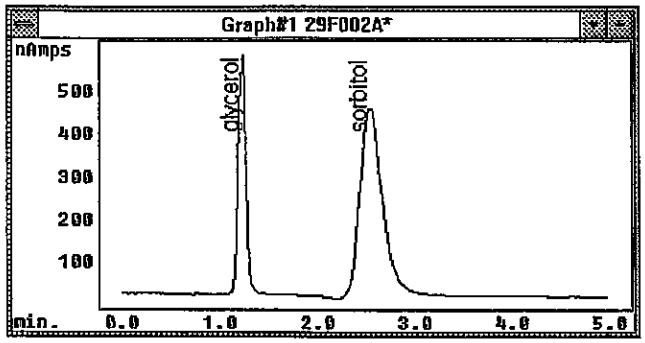

Figure 2. Separation of toothpaste sample. Calculated amounts (per gram of original sample) were 71 mg glycerol and 170 mg sorbitol.

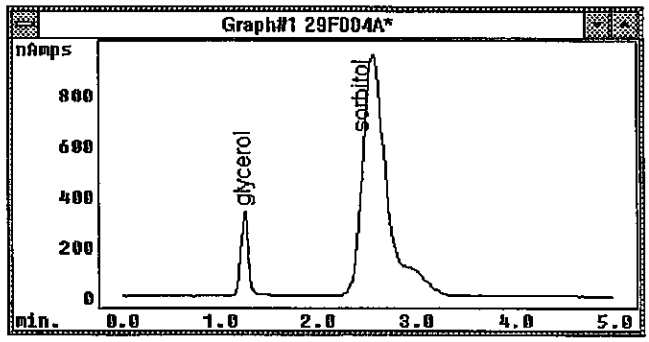

Figure 3. Separation of sorbitol-containing gum. Calculated amounts (per gram) were 41 mg glycerol and 311 mg sorbitol.

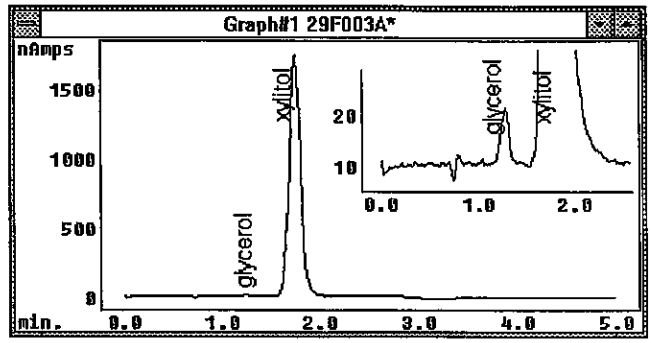

Figure 4. Separation of xylitol-containing gum. Calculated amounts (per gram) were 1.1 mg glycerol and 270 mg xylitol. Inset shows expanded scale for glycerol peak.

References

1. *P. Luo, M.Z. Luo and R.P. Baldwin, J. Chem. Educ. 70 (1993): 679-681.*
2. *BAS Application Capsule #245, LCEC of Sugars at a Copper Electrode.*
3. *D.C. Johnson and W.R. LaCourse, Anal. Chem. 62 (1990): 589A-597A.*
4. *D.C. Johnson, D. Dobberpuhl, R. Roberts and P. Vandeberg, J. Chromatogr. 640 (1993): 79-96.*